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Research Note—

Effect of a Selected *Lactobacillus* spp.–Based Probiotic on *Salmonella enterica* Serovar Enteritidis–Infected Broiler Chicks

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SUMMARY. The effect of a *Lactobacillus* spp.–based probiotic (FM-B11^{TM}) on *Salmonella enterica* serovar Enteritidis (SE) recovery was evaluated in liquid (Expt. 1) and lyophilized (Expt. 2) forms in two separate experiments with two trials each. For each trial, 80 broiler chicks were randomly allocated into two treatments: control and probiotic culture. All chicks were challenged with SE ($\sim 10^4$ colony-forming units [cfu]) upon arrival at our laboratory. In both experiments, probiotic culture was administered in the drinking water for 3 consecutive days at a final concentration of approximately 10^6 cfu/ml, beginning 1 hr after SE challenge. Cecal tonsils were aseptically removed at 24 and 72 hr postchallenge, followed by enrichment and plating on xylose lactose deoxycholate (XLD) agar for the presence or absence of *Salmonella*-typical colonies. In Expt. 1, a significant reduction ($P < 0.05$) in SE-positive samples was observed in both trials at 24 and 72 hr postchallenge. Additionally, in Expt. 2, the lyophilized probiotic decreased ($P < 0.05$) SE recovery at both 24 and 72 hr postchallenge compared with the control group in trial 1. In trial 2, SE evaluation was performed only at 72 hr after challenge and fewer ($P < 0.001$) treated samples were positive for SE. Results showed that application of either liquid or lyophilized probiotic culture in the drinking water for 3 consecutive days can help to reduce SE recovery from young birds, although further research is needed to elucidate the mechanism of this response.

RESUMEN. *Nota de Investigación*—Efecto de un probiótico basado en *Lactobacillus* spp seleccionado, sobre pollos de engorde infectados con *Salmonella enterica* serovar Enteritidis.

El efecto de un probiótico (FM-B11^{TM}) basado en *Lactobacillus* sobre *Salmonella enterica* serovar Enteritidis (SE) fue evaluado en dos experimentos separados usando dos formas de presentación: liquido (experimento 1) y liofilizado (Experimento 2). El producto se evaluó en dos experimentos separados con dos réplicas cada uno. Para cada réplica se distribuyeron al azar 80 pollos de engorde en dos tratamientos: control y cultivo probiótico. Todos los pollos fueron desafiados con *Salmonella enterica* serovar Enteritidis ($\sim 10^4$ Unidades Formadoras de Colonia [UFC]) al momento de llegar al laboratorio. En ambos experimentos, el cultivo probiótico fue administrado en el agua de bebida por 3 días consecutivos a una concentración final de aproximadamente 10^6 UFC/ml, empezando una hora después del desafío con SE. Las tonsilas cecales se removieron en forma aséptica 24 a 72 horas después del desafío; seguido de enriquecimiento y siembra en agar xilosa - lactosa deoxicolato (XLD, por su sigla en Inglés) para determinar la presencia o ausencia de colonias típicas de *Salmonella*. En el experimento 1 se observó una disminución significativa ($P < 0.05$) de muestras positivas a SE en ambos grupos a las 24 y las 72 horas postdesafío. Adicionalmente, en el experimento 2, el probiótico liofilizado disminuyó significativamente ($P < 0.05$) la recuperación de SE tanto a las 24 como a las 72 horas postdesafío comparado con el grupo control en el ensayo 1. En el ensayo 2, la evaluación de SE fue realizada únicamente a las 72 horas postdesafío y un menor número de muestras tratadas fueron positivas a SE ($P < 0.001$). Estos resultados muestran que la aplicación de un cultivo probiótico en forma líquida o liofilizada en el agua de bebida por tres días consecutivos puede ayudar a reducir el aislamiento de SE de aves jóvenes, aunque se requiere de investigaciones posteriores para aclarar el mecanismo de esta respuesta.

Key words: chick, probiotic, *Salmonella*, *Lactobacillus*

Abbreviations: CE = competitive exclusion; cfu = colony-forming units; GRAS = generally recognized as safe; LIQ = liquid probiotic culture; LYO = lyophilized probiotic culture; MRS = de Man-Rogosa Sharpe; NA = nalidixic acid; NPIP = National Poultry Improvement Plan; NO = novobiocin; SE = *Salmonella enterica* serovar Enteritidis; ST = *Salmonella enterica* serovar Typhimurium; TSB = tryptic soy broth; XLD = xylose lactose deoxycholate agar

Despite advances in the treatment of infectious diseases, pathogenic microorganisms, including *Salmonella*, are an important threat to health worldwide (22). The FoodNet surveillance program has estimated about 1.4 million cases of salmonellosis occur annually in the United States, resulting in ~16,000 hospitalization and >500 deaths (15). Poultry products have been implicated as the most common vehicle of salmonellosis transmission. Poultry often become infected by consuming contaminated feed, by cross-contamination in brooding houses, or during slaughter and processing (12).

Recent restrictions on the use of some antimicrobials as growth promoters in animal production have pressured the poultry industry to look for alternatives that can continue to provide performance benefits. Probiotic cultures have recently been evaluated for this purpose with some success (4,8). However, since Nurmi and Rantala (18) proposed that competitive exclusion (CE) could be used as a method to prevent *Salmonella* infection, numerous researchers have reported the ability of live bacterial cultures (2,16,17) and probiotic organisms (3,14,21) to reduce colonization of opportunistic microorganisms in the gastrointestinal tract by competition for

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Table 1. Therapeutic effect of *Lactobacillus* spp.–based probiotic (FM-B11™) in liquid form (LIQ) administered in drinking water for 3 consecutive days on *Salmonella enterica* serovar Enteritidis (SE) recovery in broiler chicks.

		Probiotic concentration in the drinking water (cfu/ml)			SE-positive samples/total (%)	
Trial	Treatments	24 hr	48 hr	72 hr	24 hr	72 hr
1[A]	Control	0	0	0	14/20 (70)	13/20 (65)
	LIQ	2.0×10^6	4.0×10^6	3.0×10^6	1/20 (5)*	5/20 (25)**
2[B]	Control	0	0	0	13/20 (65)	12/20 (60)
	LIQ	9.0×10^6	6.0×10^6	5.0×10^6	4/20 (20)*	4/20 (20)*

[A]6.0×10^3 cfu/bird *Salmonella* serovar Enteritidis.
[B]7.5×10^3 cfu/bird *Salmonella* serovar Enteritidis.
*Values in the same column are significantly different from the control value ($P < 0.01$).
**Values in same column are significantly different from the control value ($P < 0.05$).

receptor sites, stimulation of the immune system, and production of some active antimicrobial substances (20).

Probiotic organisms are live microbial-feed supplements that exert beneficial effects on the host by improving the microbiologic balance of the intestine (9). Lactic acid bacteria are considered to be optimal probiotic bacterial candidates because they are generally recognized as safe (GRAS). Unpublished data from our laboratory has shown that probiotic organisms contained in the culture used in these experiments inhibit the growth of food-borne pathogens under *in vitro* conditions. The aim of this study was to evaluate the therapeutic effect of a commercial probiotic (FM-B11™, IVESCO, LLC, Iowa Falls, IA) in both liquid and lyophilized form when provided in the drinking water for 3 consecutive days on *Salmonella* Enteritidis (SE) colonization in day-of-hatch broiler chicks.

MATERIALS AND METHODS

Salmonella enteritidis. A primary poultry isolate of *Salmonella enterica* PT13A serovar Enteritidis (SE) was obtained from the U.S. Department of Agriculture (USDA) National Veterinary Services Laboratory. This isolate was resistant to novobiocin (NO; 25 μg/ml) and was selected for resistance to nalidixic acid (NA; 20 μg/ml) in our laboratory. For these studies, SE was grown in tryptic soy broth (TSB) at 37 C for 8 hr and passed to fresh TSB for three incubation periods. Cells were washed three times in sterile saline by centrifugation at 1864 $\times$ *g*, and the concentration was estimated with a spectrophotometer using a previously generated standard curve, to approximately 10^8 cfu/ml in sterile saline. The culture was then diluted to inoculated concentrations as described below. Concentrations of SE and *Salmonella enteritidis* serovar Typhimurium (ST) were retrospectively determined by spread plating on xylose lactose deoxycholate (XLD) agar containing NO (25 μg/ml) and NA (20 μg/ml), followed by enumeration for each experiment. Actual determined colony-forming units (cfu) for each experiment are reported.

Probiotic administration. Eleven lactic acid bacterial isolates were previously selected and have been previously described (8). This mixture, FM-B11 (IVESCO, LLC), now commercially available in both liquid and powdered (lyophilized) forms, was used for these experiments. For Experiment 1, the liquid probiotic culture (LIQ) containing 10^9 cfu/ml was diluted 10-fold in de Man-Rogosa-Sharpe (MRS) broth, then 35 ml was added to 3425 ml of fresh drinking water and given to the chicks approximately 1 hr after SE challenge. For Experiment 2, lyophilized probiotic culture (LYO) was obtained that contained $\sim 10^{11}$ cfu of viable organisms. A 1000-fold dilution (1:1000) was made (final concentration, 10^8 cfu/ml) in MRS broth, and 35 ml was added to 3425 ml of drinking water, and 35 ml of skim milk was added to the drinking water in both experiments as a stabilizer. Enumeration of viable organisms of the probiotic cultures was performed on MRS agar plates, and the final concentrations were $\sim 10^6$ cfu/ml (Tables 1 and 2).

***Salmonella* recovery.** Briefly, cecal tonsils were aseptically removed and incubated for 24 hr in tetrathionate broth at 37 C. After incubation, a sample of broth was streaked for isolation on XLD agar plates containing NO/NA antibiotics as described above and was incubated for an additional period of 24 hr at 37 C. Following incubation, agar plates were evaluated for the presence or absence of typical antibiotic-resistant *Salmonella* colonies.

Experiment 1. This experiment was replicated in two separate trials. Day-of-hatch broiler chicks were obtained from a commercial hatchery and placed in brooder batteries located in an isolation room in the Poultry Health Laboratory at the University of Arkansas. Before the start of the experiment, five chicks and feed were cultured for *Salmonella*, using a previously described procedure (1). Chicks were provided with unmedicated chicken starter feed *ad libitum* and fresh water daily, with or without probiotic treatment, according to the experimental design. In trial 1, 80 broiler chicks were randomly assigned to either the control or probiotic treatment (LIQ). Chicks were individually intubated for gavage with 6.0×10^3 cfu of SE before placement in brooder batteries. Probiotic was provided for 3 consecutive days beginning 1 hr after *Salmonella* challenge. For trial 2, 80 chicks were treated as described in trial 1 and challenged with 7.5×10^3 cfu/bird SE. In both trials, 20

Table 2. Therapeutic effect of a *Lactobacillus* spp.–based probiotic (FM-B11™) in lyophilized form (LYO) in the drinking water for 3 consecutive days *Salmonella enterica* serovar Enteritidis (SE) recovery in broiler chicks.

		Probiotic concentration in drinking water (cfu/ml)			SE-positive samples/total (%)	
Trial	Treatments	Day 1	Day 2	Day 3	24 hr	72 hr
1[A]	Control	0	0	0	16/20 (80)	16/20 (80)
	LYO	7.0×10^5	5.0×10^5	2.0×10^6	10/20 (50)*	9/20 (45)*
2[B]	Control	0	0	0	NE[C]	24/25 (96)
	LYO	2.0×10^6	1.0×10^6	2.0×10^6	NE	11/25 (44)**

[A]1.0×10^4 cfu/bird *Salmonella* serovar Enteritidis.
[B]5.0×10^3 cfu/bird *Salmonella* serovar Enteritidis.
[C]NE = not evaluated.
*Values in the same column significantly different from the control value ($P < 0.001$).
**Values in the same column significantly different from the control value ($P < 0.05$).

chicks per group were humanely killed at 24 or 72 hr postchallenge according to the National Poultry Improvement Plan (NPIP) guidelines (19).

Experiment 2. Two trials were performed at different times using the same model as used in Experiment 1. For this experiment, probiotic treatment (LYO) was added to drinking water 1 hr after *Salmonella* challenge and continued for 3 days. In trial 1, the SE challenge was 1.0×10^4 cfu per chick, and in trial 2, the challenge was 5.0×10^3 cfu per chick. Trial 2 used only 25 chicks in each group, instead of 40, as described in previous trials.

Statistical analysis. Chi-square analysis was performed in each experiment to determine significant differences ($P \leq 0.05$) between groups in SE recovery rate (7).

RESULTS

Experiment 1. In Experiment 1, a significant reduction ($P < 0.01$) in *Salmonella* recovery was observed among chicks that received LIQ (1/20; 5%) compared with the control group (14/20; 70%) after 24 hr of treatment (Table 1). At 72 hr after challenge, significantly fewer samples ($P < 0.05$) were positive for SE in the LIQ group (5/20; 25%) in comparison with the control group (13/20; 65%). Likewise, in trial 2, a reduction ($P < 0.01$) of SE recovery was observed among LIQ-treated chicks compared with untreated chicks at both 24 and 72 hr.

Experiment 2. In the first trial of Experiment 2 (Table 2), the number of SE-positive samples was significantly lower ($P < 0.05$) among chicks treated with LYO than among the control group at 24 and 72 hr postinfection. In trial 2, *Salmonella* evaluation was performed only at 72 hr postchallenge, and the colonization of SE was reduced ($P < 0.001$) among LYO-treated broiler chicks in comparison with the control group at this time point.

DISCUSSION

Many reports support the benefits of administering normal microflora of healthy adult poultry to young chicks to prevent infections (2,17,21). Organisms present in the *Lactobacillus*–based probiotic (FM-B11) include live, poultry-origin, lactic acid bacteria: *Lactobacillus fermentum*, *Lactobacillus helveticus*, *Lactobacillus paracasei*, *Lactobacillus salivarius*, and *Pediococcus parvulus* (based upon 16-s RNA sequencing). Data obtained in this report suggest that the combination of these probiotic strains may be used as a tool to significantly reduce SE colonization in broiler chicks from day of hatch. Furthermore, the supplementation of this culture for 3 consecutive days continued to maintain reduced levels of SE recovery from cecal tonsils.

Administration of a probiotic culture may modify the ecology of the gastrointestinal tract during the first days of a chick's life, a time that is considered an open window for the establishment of pathogens, such as *Salmonella*. Neonates are born with an almost a sterile gastrointestinal tract, but microorganisms present in the environment after birth rapidly begin colonize (5). Although lactic acid bacteria are normal inhabitants of the gastrointestinal tract, their presence follows a succession with *Lactobacillus delbrueckii* as the major species at day 3, *Lactobacillus acidophilus* and *Weissella* spp. dominating at day 7, and *Lactobacillus crispatus* predominating from days 14–49. Lastly, *L. salivarius* appeared at day 49 (13). In addition, Guan *et al.* (6) observed that *L. acidophilus* and *L. salivarius* appeared in developmental succession, whereas other species, such as *Lactobacillus reuteri* and *Lactobacillus johnsonii* were consistently detected.

Reduction of pathogens has been observed by others following administration of probiotic cultures. Pascual *et al.* (19) observed that oral administration of *L. salivarius* strain probiotic organism in day-old Leghorn chicks caused no *Salmonella* to be recovered after 21 days. The same results were observed when the probiotic strain was administered through the feed and drinking water apart from oral gavage. In other studies, application of lactose and a probiotic culture (10^9 cfu/gram) during the grow-out period not only improved performance in broiler chicks but also reduced coliforms in the cecum at 10 days of age (11). Oral gavage with a lyophilized CE product in broiler chicks reduced coliforms in the small intestine, large intestine, and cecum at days 7 and 14 (9). However, to our knowledge, this is the first study to evaluate both liquid and lyophilized forms of the same culture for reduction of a pathogen.

In conclusion, the administration of either a liquid or a lyophilized *Lactobacillus*–based probiotic (FM-B11) in the drinking water may help to reduce the incidence of *Salmonella* recovery in broiler chicks. Further research is necessary to elicit the specific mechanisms of the protection benefits provided by probiotic culture.

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